

MILK LEUKOCYTE ADHESION MOLECULES IN RESPONSE TO *Curcuma longa* PLUS α -TOCOPHEROL AND SELENIUM IN MASTITIC RIVERINE BUFFALOESReena Mukherjee¹, R.K. Jadhav², Nitika Sharma³, B.D. Sahu² and U.K. De⁴

ABSTRACT

Somatic cell count (SCC), total bacterial count (TBC), L selectin and CD 18 on milk leukocytes was studied in buffaloes inflicted with intramammary infection in response to *Curcuma longa* (*C. longa*, CL) and vitamin E plus selenium (group II), Enrofloxacin (group III) and sterile PBS (group IV). Significant reduction ($P < 0.05$) in SCC, TBC was observed in post-treated buffalo cows. The mean fluorescent intensity (MFI) of L selectin increased significantly ($P < 0.05$) in Group 2 post-treated cows; however, there was no reduction in CD 18 counts in this group. The results suggest that *Curcuma longa* (CL) possesses antibacterial, anti-inflammatory and immunomodulatory properties. In the present study the biological activity of the CL and vitamin E plus selenium at standardized dose against mastitis in buffalo cows is reported for the first time. Development of alternative therapy is an option for livestock farmers who are not allowed to use allopathic drugs under certain farming system.

Keywords: CD 18, *Curcuma longa*, L selectin, mastitis, vitamin E

INTRODUCTION

Considerable progress has been made through extensive research on bovine mastitis, still it continues to be the most frequent and costly disease of dairy animals (Ruegg, 2010). Leukocyte migration from the central pool into the infected udder depends on the activation of leukocyte adhesion molecules (LAM), however the expression of LAM like L selectin is generally lower during periparturient period and mastitis (Paape *et al.*, 2002). Antibiotics are used for the treatment of mastitis, but it has got few limitations, like development of antimicrobial resistance and antibiotic residue in milk. Few antibiotics also reduce the functional activities of the immune cells. *Curcuma longa* (CL) or turmeric belongs to *Zingiberaceae* family, the rhizomes possess antimicrobial, anti-inflammatory, antioxidant and immunomodulatory properties (Menon and Sudhir, 2007). Antimicrobial activity of *Curcuma longa* against *S. aureus*, *S. epidermis*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* was observed by many researchers (Singh *et al.*, 2002, Han yang, 2005). Micronutrients and vitamins like

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Selenium and Vitamin E helps in reducing severity and duration of clinical mastitis by alleviating inflammatory metabolites from the site of infection and by improving leukocyte activities (Politis *et al.*, 2004; Smith *et al.*, 1997, Mukherjee, 2008). There are no reports regarding the immunotherapeutic potential of CL against bovine mastitis. The present study, therefore, is an effort to evaluate the potential of CL along with selenium and vitamin E on the somatic cell count (SCC), total bacterial count (TBC) and milk LAM from the lactating buffalo cows inflicted with intramammary infection.

MATERIALS AND METHODS

Hydro-methanolic extract of *C. longa* (CL) was prepared as per (Peach and Tracy, 1956). The antibiogram and MIC of the extract was done as per standard method (NCCLS, 1997) against *Staphylococcus aureus*, CNS, *Streptococcus agalactiae*, *Streptococcus uberis* and *Coliform bacilli*. The dose was determined on the basis of mean MIC of the extract against the pathogens

Twenty-four crossbred lactating buffaloes were divided in four equal groups. Buffalo cows of Group 1, selected where milk SCC < 0.5 million cells /ml of milk, served as control. Eighteen mastitic animals selected formed Groups 2, 3 and 4 positive for intramammary infection were taken for the drug trial, selection criteria being the SCC > 0.7 million cells /ml of milk and milk secretions positive for pathogenic isolate. Buffalo cows of Group 2 received 150 mg of sterile CL extract per teat after reconstituting in 5 ml warm PBS by i/mam route with a sterile antibiotic dispensing canula for 5 days. Vitamin E plus selenium was also given at the rate of 10 ml per buffalo by intramuscular route on day 0 only. Group 3 buffaloes were treated

with enrofloxacin at the dose rate of 3 mg per kg body weight by i/m route for 5 days or till clinical recovery. Group 4 animals received 5 ml of sterile PBS for 5 days by i/mam route. SCC and TBC of the milk samples were done as per the standard method (Schalm *et al.*, 1971) (Balows *et al.*, 1991). The identification and biochemical characterization of causative organism in collected milk samples was carried as per (Balows *et al.*, 1991). SCC and TBC were done on day '0' and thereafter on 2, 10 days PT. Milk leukocytes were isolated as per the method describe by Daley *et al.* (1991) for enumeration of CD 18 and L- Selectin in milk PMNs. About 100 µl of milk cell suspension was incubated in the dark with 1 µl of primary antibodies against CD 18 and L- Selectin for 30 minutes at room temperature. CD 18 and L-Selectin was done by using commercial kit (CD 18 ,Clone- BAQ30A, Isotype- IgG1, VMRD, Inc. Pullman, WA, USA and L-Selectin, Clone- DU1-29, Isotype- IgG1, VMRD, Inc. Pullman, WA, USA) as per method described by Soltys and Quinn (1999).

The data were analyzed applying one-way analysis of variance (ANOVA) to determine the level of significance between the groups, and Duncan's multiple range test (DMRT) was applied to determine the level of significance within the group at different time intervals by using an SPSS 10.1 software package.

RESULTS AND DISCUSSION

There were no differences in SCC and TBC in the milk samples isolated from healthy cows at different time intervals of the study period. The SCC and TBC in Group 2 and Group 3 significantly decreased ($P < 0.05$) on day 7 and day 15 (Table 1). Out of 14 milk samples collected

from diseased buffalo cows, the organisms isolated were *Staphylococcus aureus* (7%), CNS (56%), *Streptococcus agalactiae*. (3%), *Streptococcus uberis* (9%), *Coliform bacilli* (14%), no growth (11%). Mean fluorescent intensity of L selectin was lower in mastitic cows ($P < 0.05$) before treatment. However, the expression increased (37.42%) in Group 2 cows on day 7, whereas non-significant increased expression of L selectin was recorded in Group 3 posttreated animals (Table 2). Contrary to values of L selectin, the expression of CD18 was higher in the PMNs isolated from mastitic cows both before and after treatment ($P < 0.05$). However, it reduced significantly ($P < 0.05$) in Group 3 (42.26%) on day 7 as compared to pretreatment values and Group 2 (Table 2).

Mastitis is one of the major causes of economic loss in dairy cattle. Once established in the udder, it impairs alveolar function, reduces milk yield and has a deleterious effect on milk composition, including increased milk SCC and bacterial load. In the present study the SCC and TBC were higher in mastitic buffalo cows before treatment ($P < 0.05$). Regulation of PMNs migration from the central pool to the infected site is of great importance for host defense against mastitis (Paape *et al.*, 2002). For appropriate elimination of pathogen the effectiveness of the drug and optimum functioning of the hosts' immune system is required. In and around the calving period there is down-regulation of selectins, which affects migration of leukocytes into the udder parenchyma and its functional activities (Lee and Kehrli, 1998; Paape *et al.*, 2002). Leukocyte activities can be modulated by number of specific and non-specific mediators (Smith, 1994). Antibiotics are used to treat mastitis, but they are not effective in removing the inflammatory metabolites from the tissue milieu and also contaminate the milk (Erskine, 2000).

In the present drug trial significant reduction of SCC and TBC was observed in Group 2 treated with CL along with i.m. administration of vitamin E and selenium; however, the therapy enhanced the expression of L selectin and did not reduce the MFI of CD 18 in post- treated buffalo cows. On the contrary, the expression of CD 18 reduced significantly in Group 3 cows treated with enrofloxacin.

C. longa, commonly known as 'turmeric', is widely used as a spice in Indian cuisine. The rhizome possess several medicinal properties. Curcumin is the main phytochemical principal of CL attributing a wide array of biological activities such as antioxidant, anti-inflammatory, wound-healing, antifungal and antibacterial activity (Singh *et al.*, 2002). Singh *et al.* (2002) observed inhibition of growth of pathogenic *S aureus* with CL extract. Menon and Sudhir (2002) suggested that the therapeutic effect of CL could be due to its anti-inflammatory and antioxidant properties. Udder health in lactating animals is also influenced by the dietary status, supplementation of selenium and vitamin E in feed of lactating dairy cows was found to improve leukocyte migration, phagocytosis and intracellular killing respectively (Smith *et al.*, 1997). It has been observed that supplementation of micronutrients and vitamin E in feed helps in reducing severity and duration of clinical mastitis and helps in alleviating the acute phase response from the site of infection (Politis *et al.*, 2004). There are several reports on the therapeutic effect of vitamin E and selenium against mastitis; however, its synergistic effect with CL against mastitis, particularly the leukocyte activities, are undefined. In conclusion, this study represents an initial investigation into the synergistic effect of vitamin E plus selenium and CL on the activity of L selectin, CD 18, SCC and TBC in mastitic buffalo cows.

Table 1. Somatic cell count (SCC) 1×10^5 cells / ml and total bacterial count (TBC) 1×10^3 cells / ml of milk in response to the treatment with *C. longa* plus Vitamin E and selenium (Group 2) and Enrofloxacin (Group 3) and sterile PBS (Group 4) mastitic cows and in normal healthy cows (group I) (mean \pm SE).

Groups	Days post treatment		
	Day 0	Day 7	Day 15
SCC			
Gr I	2.79 \pm 1.22 ^x	3.02 \pm 1.26 ^x	3.09 \pm 1.14 ^x
Gr II	8.87 \pm 1.09 ^{a,y}	5.26 \pm 1.04 ^{b,y}	5.09 \pm 1.16 ^{c,y}
Gr III	9.07 \pm 1.01 ^{a,y}	5.03 \pm 1.17 ^{b,z}	5.07 \pm 1.05 ^{b,y}
Gr IV	8.53 \pm 1.33 ^{a,y}	8.12 \pm 1.72 ^{a,y}	7.46 \pm 1.96 ^{a,y}
TBC			
Gr I	0.27 \pm 1.11 ^{a,w}	0.26 \pm 1.23 ^{a,w}	0.27 \pm 1.21 ^{a,w}
Gr II	2.86 \pm 1.21 ^{a,x}	1.09 \pm 1.07 ^{b,x}	0.82 \pm 1.12 ^{c,x}
Gr III	3.06 \pm 1.73 ^{a,x}	0.98 \pm 1.05 ^{b,y}	0.58 \pm 1.79 ^{c,y}
Gr IV	2.75 \pm 1.66 ^{a,x}	2.53 \pm 1.95 ^{a,x}	2.21 \pm 1.59 ^{a,x}

*Values with different superscripts in each rows (a, b) and each column (w, x, y) differ significantly (P<0.05).

Table 2. Expression of L selectin and CD 18 in response to treatment with *C. longa* plus vitamin E and selenium (Group 2) and enrofloxacin (Group 3) and sterile PBS (Group 4) mastitic cows and in normal healthy cows (group I) (mean \pm SE).

Group of cows	L selectin on milk	PMNs	CD 18 in milk PMNs	
	Day 0	Day 8	Day 0	Day 8
Group 1	4.66 \pm 1.37 ^x	4.59 \pm 1.29 ^x	5.93 \pm 1.49 ^x	5.84 \pm 1.41 ^x
Group 2	1.99 \pm 1.52 ^{y,a}	3.18 \pm 1.16 ^{y,b}	8.63 \pm 1.68 ^{a,y}	7.81 \pm 1.17 ^{b,x}
Group 3	2.01 \pm 1.17 ^y	2.22 \pm 1.09 ^z	8.28 \pm 2.03 ^{a,y}	5.82 \pm 1.20 ^{b,y}
Group 4	2.26 \pm 1.32 ^y	2.15 \pm 1.44 ^y	8.35 \pm 2.03 ^{a,y}	7.25 \pm 1.87 ^{a,y}

*Values with different superscripts in each rows (a, b) and each column (x, y, z) differ significantly (P<0.05).

The results indicate the enhanced cellular defense of the diseased mammary gland and subsequently reduction in infection and inflammation.

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